

ANTIPSYCHOTIC ACTIVITY OF *AFRAMOMUM MELEGUETA*(ROSCOE) K. SCHUM (ZINGIBERACEAE) AND *BIDENS PILOSA* L. (COMPOSITAE) IN MALE MICE

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ABSTRACT

Mental disorder features unhealthy situations such as sleep disturbance, social withdrawal and impaired role functioning, all of which are treated with herbal formulations, by traditional healers. *Aframomum melegueta* and *Bidens pilosa* are medicinal plants applied traditionally to treat psychosis. Hence, this study investigated the antipsychotic effect of the two plants in ketamine-induced psychosis in mice. Pulverised plant samples consisting of *Aframomum melegueta* seeds and leaves, and stem of *Bidens pilosa* were macerated in absolute methanol for 72 h. Extracts were evaluated in mice for hyper-locomotion in open field and stereotype behaviour in transparent chamber models. Statistical analysis was done using one-way ANOVA at $p < 0.05$. *Aframomum melegueta* seeds, and *B. pilosa* leaves at 500 mg/kg and *B. pilosa* stem at 250 mg/kg, decreased hyperactivity in mice, when compared to the negative control group (ketamine only). Furthermore, the highest dose (500 mg/kg) of *Aframomum melegueta* seeds, *B. pilosa* leaves and 250 mg/kg of *B. pilosa* stem gave total tranquilising effect at 60 min into the experiment. Findings from this study revealed that seeds of *Aframomum melegueta*, and the leaves as well as stem of *Bidens pilosa* possess constituents responsible for the treatment of psychosis. This in turn justifies the traditional use of both plants in the treatment of mental illness. Different ratios of both plants under study may be combined as formulations and tested in future research, as a step towards drug development.

INTRODUCTION

Psychosis is described as the unification of psychological symptoms resulting in a loss of contact with reality [1]. Victims of Psychosis manifest unhealthy situations such as anxiety, sleep disturbance, social withdrawal, lack of motivation and impaired role functioning. This illness may occur due to metabolic and schizoaffective disorder, drug intoxication, bipolar disorder and, in rare cases, major depression among others [2]. Psychotic episodes can either be primary or secondary. The disorder is considered primary if it results from a psychiatric condition and secondary if it is caused by a medical condition or drugs [3]. One main theory that explains psychosis is the dopamine theory. According to this theory, the brain's limbic and subcortical D₂ receptors contribute to the positive symptoms of psychosis when overactive. Negative and cognitive symptoms of the illness could, however, result from decreased activity of dopaminergic D₁ receptors in the prefrontal cortex [4]. Some psychotic symptoms are linked to genetic and environmental variables while others are categorised as abnormalities in neurodevelopment and are thought to arise in the uterus. Drug misuse, stress, trauma, medicine, and other environmental factors are a few of the factors that could result in psychosis [5].

The drugs known as antipsychotics are used to treat psychotic disorders as well as other mental and emotional diseases. They are typically introduced slowly and at low doses, and then adjusted upward as necessary [6]. Symptoms of psychosis, such as hallucinations, depression and disordered cognition and behaviour, can be effectively treated with antipsychotic drugs. However, they are inadequate in treating the detrimental effects of psychosis [7], and extrapyramidal disturbances have been linked to antipsychotic drug adverse effects [8].

Two categories of antipsychotics, typical and atypical

antipsychotics are known. The typical antipsychotics are linked to blockage of dopamine receptors, whereas the atypical have a higher affinity for serotonin receptors (5HT_{2A}) than dopamine receptors [9]. Haloperidol, fluphenazine, chlorpromazine, are commonly used typical antipsychotics. Clozapine, olanzapine and risperidone, are a few examples of atypical antipsychotics [9].

Obtaining modern drugs for treating psychotic disorders is a costly affair, which makes it challenging for those in the lower economic strata to access them. Moreover, the use of modern antipsychotic drugs often results in various adverse effects such as seizures, postural hypotension, muscular rigidity, resting tremors, and tardive dyskinesia. In contrast, herbal drugs are easily available and relatively affordable, making them a viable alternative for treating psychosis. Herbal medicines can provide a means of antipsychotic medications that have minimal side effects.

Herbalists have extolled the health benefits of several plants, but only a tiny number of these plant species have been shown to be scientifically effective in the treatment of certain ailments. In an ethnobotanical survey carried out by our team, [10], *Aframomum melegueta* seeds and *Bidens pilosa* leaves and stem were part of the recipes used by traditional healers to treat mental illness. However, despite the potential benefits, the antipsychotic properties of these two plants have not been documented. Therefore, this study investigated the antipsychotic potential of *Aframomum melegueta* seeds and *Bidens pilosa* leaves and stem.

RESULTS

Locomotion Activity

The three doses of *Aframomum melegueta* seeds and *Bidens pilosa* leaves and stem reduced the number of lines crossed by

the animals, with 500 mg/kg showing more activity except for *Bidens pilosa* stem, which showed more activity at 250 mg/kg. The reduction in hyperactivity of the extract is similar to those treated with distilled water. Haloperidol (0.5 mg/kg) gave a significant reduction in the number of lines crossed when compared to group administered ketamine (20 mg/kg). The graphs showing number of lines crossed against the different doses of extracts from *A. melegueta* seeds, *B. pilosa* leaves and stem are shown in Figures 1, 2 and 3, respectively.

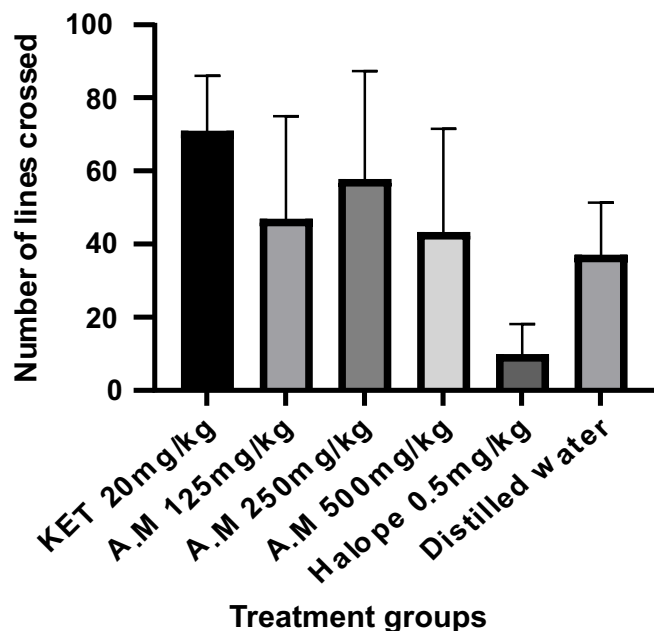


Figure 1: Effect of different doses of *Aframomum melegueta* seeds (125 mg/kg - 500 mg/kg), haloperidol (0.5 mg/kg) against ketamine induced hyper-locomotion of mice in open field chamber. One way ANOVA showed ***P = 0.0018 between treatments. KET- Ketamine, A.M- *Aframomum melegueta*, Halope- Haloperidol

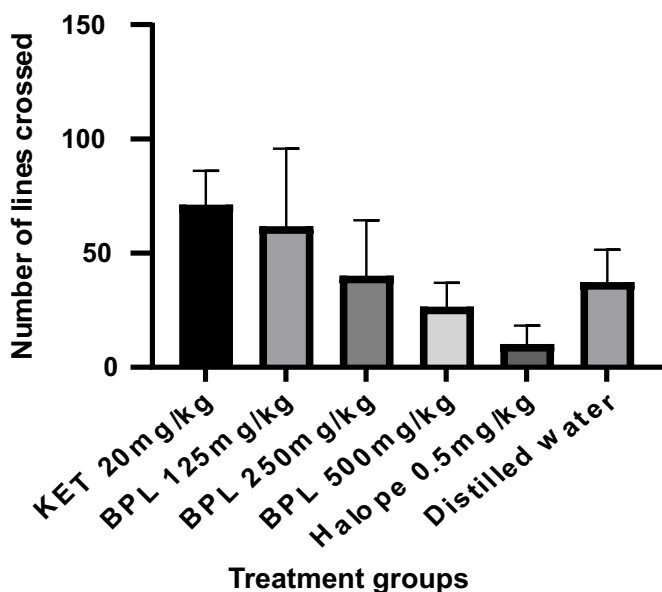


Figure 2: Effect of different doses of *Bidens pilosa* Leaf (125 mg/kg - 500 mg/kg), haloperidol (0.5 mg/kg) against ketamine induced hyper-locomotion of mice in open field chamber. One way ANOVA showed ***P = 0.0001 between treatments. KET- Ketamine, BPL- *Bidens pilosa* Leaves, Halope- Haloperidol

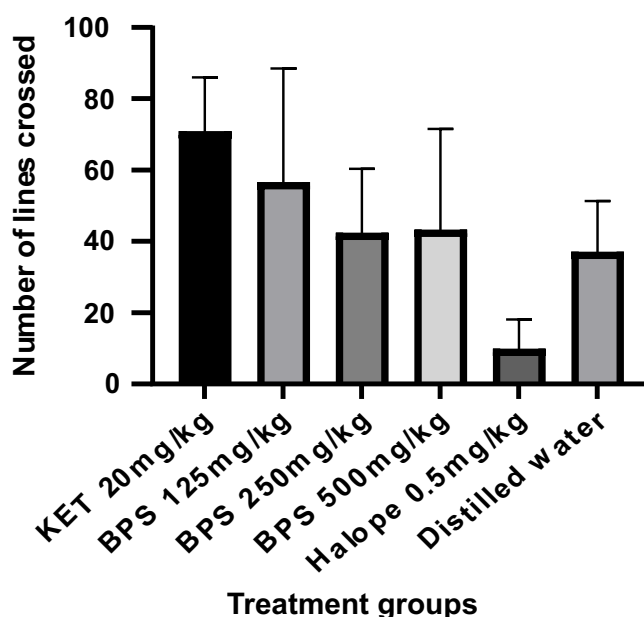


Figure 3: Effect of different doses of *Bidens pilosa* stem (125 mg/kg - 500 mg/kg), haloperidol (0.5 mg/kg) against ketamine induced hyper-locomotion of mice in open field chamber. One-way ANOVA analysis showed significant difference at ***P = 0.0009 among all treatments. KET - Ketamine, BPS- *Bidens pilosa* stem, Halope- Haloperidol.

Stereotype Behaviour

The dose of 250 mg/kg and 500 mg/kg of *Aframomum melegueta* seeds extract reduced the stereotype behaviour induced by ketamine (Figure 4). The 500 mg/kg dose of *Bidens pilosa* leaves showed more tranquilising effect in the animals at 60 min of the experiment (Figure 5). In *Bidens pilosa* stem, the dose of 250 mg/kg gave a better calming effect as compared to other doses of the extract as shown in Figure 6.

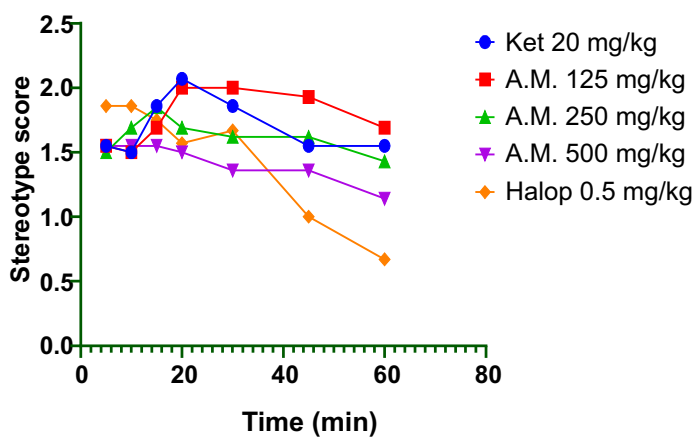


Figure 4: Effect of methanol extract of *Aframomum melegueta* seeds (125 mg/kg- 500 mg/kg), haloperidol (0.5 mg/kg), on ketamine-induced stereotype behaviour of mice in transparent observation chamber (**P = 0.0185, Bartlett's posthoc test) Ket; ketamine, AM: *Aframomum melegueta*, Halop: Haloperidol

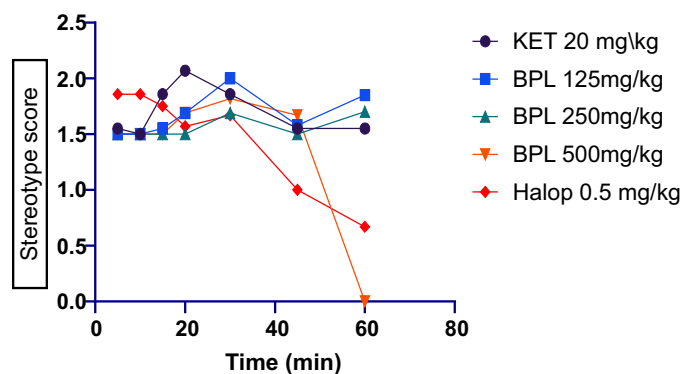


Figure 5: Effect of methanol extract of *Bidens pilosa* leaves (125 mg/kg- 500 mg/kg), haloperidol (0.5 mg/kg) against ketamine-induced stereotypy behaviour of mice in transparent observation cages
KET- Ketamine, BPL- *Bidens pilosa* Leaves, Halope- Haloperidol

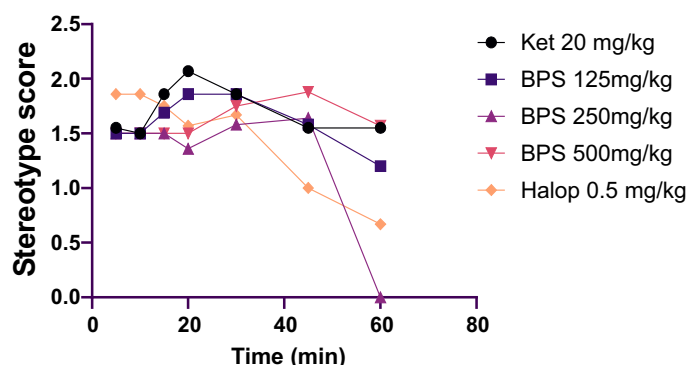


Figure 6: Effect of methanol extract of *Bidens pilosa* stem (125 mg/kg- 500 mg/kg), haloperidol (0.5 mg/kg) against ketamine-induced stereotypy behaviour of mice in transparent observation cages
KET- Ketamine, BPS- *Bidens pilosa* stem, Halope- Haloperidol

DISCUSSION

Mental maladies have become highly widespread due to determined lifestyle, urbanisation, and stressful environment. Locomotion activity in animal models is a behavioural indicator that has been linked to the restlessness and disorganised behaviour observed in cases of psychosis [11]. Due to its comparative simplicity in measurement, locomotor activity assessment has been extensively employed to model the positive symptoms associated with schizophrenia. A partial understanding exists regarding the neurotransmitter mechanisms in the brain that contribute to psychotic symptoms, and it is possible to induce similar neurotransmitter alterations in rodents through drug administration. The historical assumption of subcortical hyper-dopaminergic in schizophrenia largely stemmed from the neuropharmacological effects of antipsychotic medications [12]. The idea behind testing locomotor hyperactivity is founded on the notion that increased dopamine activity in rodents result in heightened motor behaviour, which can manifest as horizontal locomotion, rearing, or, at higher doses, repetitive behaviours [13].

The locomotion test aims to assess the antipsychotic property of potential drugs, by measuring their ability to reduce hyperactivity in animals. From the result, it was observed that all three doses of *Aframomum melegueta* reduced the number of lines crossed by the animals. Hence, they reduced the hyperactivity of the animals when compared to the ketamine

group. The 500 mg/kg showed more activity when compared to other doses. The reduction in hyperactivity of the extract was similar to those treated with water alone. However, the reduction was not as significant as that of the group tested with haloperidol. Varying doses of *B. pilosa* leaves (125 mg/kg- 500 mg/kg) demonstrated a dose-dependent response, though in a reverse order. The highest dose (500 mg/kg) exhibited increased activity, although not surpassing that of haloperidol. *B. Pilosa* stem indicated that 250 mg/kg dose was effective against hyper-locomotion activity. It is worth mentioning that all dosages demonstrated efficacy in decreasing the quantity of lines crossed.

The stereotype model is another paradigm used to test positive symptoms of psychosis. Stereotypy refers to repetitive behaviour and has been observed in individuals with psychosis, as well as in various pharmacological models of psychosis in both humans and animals [14]. The particular behaviours exhibited may differ based on the specific drug or animal employed. These behaviours encompass repetitive actions such as licking, chewing, grooming, head movements, sniffing, circling, or climbing, among others [15]. The intensity of stereotypy can be assessed through either direct observation or by utilising software programmed with pre-defined criteria [16].

From the experiment, the lowest dose (125 mg/kg) of *Aframomum melegueta* did not reduce the repetitive behaviour to a significant effect. The dose of 250 mg/kg of the extract reduced the stereotype behaviour at 20, 30, and 60 minutes. Additionally, 500 mg/kg reduced the stereotype behaviour at 15, 20, 30, 45 and 60 minutes. The 250 mg/kg dose of *Bidens pilosa* leaves at the beginning of the experiment exhibited activity against stereotype behaviour. However, the activity was seen to decrease across time of the experiment. The dose of 500 mg/kg, was shown to give a reduction in stereotype behaviour of the animals and also gave a total tranquilising effect at 60 minutes into the experiment (Figure 5). Furthermore, *Bidens pilosa* stem at 500 mg/kg gave a reduction in stereotype behaviour at the beginning of the experiment but the activity decreased across time of the experiment. However, 250 mg/kg dose gave a better reduction in the repetitive behaviour of the animals with a tranquilising effect at 60 minutes into the experiment (Figure 6).

In the evaluation of the effect of *Aframomum melegueta* seeds, *Bidens pilosa* leaves and stem extracts on ketamine-induced psychosis, the doses of 500 mg/kg for *Aframomum melegueta* seeds, *Bidens pilosa* leaves, and 250 mg/kg for *Bidens pilosa* stem, demonstrated effectiveness in reducing hyper-locomotion and stereotype behaviours. An over-activity of dopamine transmission in certain areas of the brain is associated with positive symptoms of psychosis, which can be measured using the hyper-locomotion and stereotype behaviour models. The finding in this study indicates that the extracts of *Aframomum melegueta* seeds, *Bidens pilosa* leaves and stem may reduce dopamine signals in the brain by blocking D_2 receptors, thereby alleviating the psychotic symptoms [17] in the mice. This study is in line with previous experiment in which Usman *et al.*, [18] reported that ethanol extract of *Blighia sapida* stem bark reduced apomorphine and ketamine induced stereotype behaviours and hyperactivity in animals. Furthermore, *Rhodiola rosea* root extract was reported to restore both apomorphine and dizocilpine induced prepulse

inhibition deficits in rodent models of psychosis [19].

CONCLUSION

Findings from this study revealed that seeds of *Aframomum melegueta*, and the leaves as well as stem of *Bidens pilosa* possess constituents responsible for the treatment of psychosis. This justifies the traditional use of both plants in the treatment of mental illness. Different ratios of both plants under study may be combined as formulations and tested in future research, as a step towards drug development.

MATERIALS AND METHODS

Plant collection and preparation of crude extracts

Aframomum melegueta and *Bidens pilosa* plants were collected from Ipara, Remo North, Ogun State and Goodness Estate, Arunlogun Road, Ojoo, Ibadan, Oyo State, Nigeria, respectively, in November 2022. Identification and authentication were carried out by Mr. D.P. Esimekhai at the University of Ibadan Herbarium. Voucher specimens were deposited with numbers UIH-23192 and UIH-23194, respectively.

Dried seeds of *Aframomum melegueta* as well as shade dried leaves and stem of *B. Pilosa* were crisp-dried in a regulated oven at 40°C for five minutes prior to pulverisation. Pulverised *Aframomum melegueta* (one kilogram) and *Bidens pilosa* leaf (300 g) and stem (800 g) samples were macerated in 4 L and 2.5 L each, respectively, absolute methanol for 72 h. The mixture was intermittently stirred using a glass rod to enhance extraction. Three days after, the filtrates were decanted and evaporated *in vacuo* using a rotary evaporator. Solvent remaining in concentrated extracts was removed and dried, using a regulated water bath at 50°C. The crude extracts were then stored in sterile air-tight bottles until needed for use.

Animals

Swiss albino male mice within the weight range of 18 g to 26 g were obtained from the Central Animal House, University of Ibadan. They were kept at room temperature in large transparent, plastic cages with perforated plastic-metallic covering, at the Animal house, Department of Pharmacology and Therapeutics, University of Ibadan, Nigeria. The floors of the cages were filled with shavings from wood and were replaced every three days. The mice were fed daily *ad libitum* with balanced rodent pellets; they were also given clean drinking water. The mice were acclimatised for one week before the commencement of the study.

Drug Administration

Drugs were administered to the experimented animals according to the method described by Isola *et al.*, [20]. The animals were divided into twelve (12) groups, with six animals per group. Each group of animals were treated with ketamine (20 mg/kg), haloperidol (0.5 mg/kg), distilled water (10 mL/kg) and three different doses (500 mg/kg, 250 mg/kg and 125 mg/kg) of the crude extracts from seeds of *A. melegueta*, leaves and stem of *Bidens pilosa*. The animals were re-weighed prior treatment, to determine the actual volume of drug to be administered. According to Parimalakrishnan *et al.* [21], the acute toxicity carried out for *Bidens pilosa* methanol extract revealed LD₅₀ of 3.5 g/kg indicating a safe extract. The experimental procedures were in compliance with the National Institutes of Health guide for care and use of laboratory animals.

Experimental Procedures for Psychosis

Locomotion Test

The locomotion assessment was conducted utilising the modified technique described by Arowona *et al.* [22]. The animals were administered with treated drugs (Haloperidol, Distilled water and crude extracts) and placed in transparent cages for one hour. In a one hour post-treatment, ketamine was intraperitoneally administered to each mouse to induce psychosis. After induction, mouse was immediately placed in the centre of an open field chamber, the vertical, horizontal and diagonal movements across the chambers were observed for 5 minutes and number of lines crossed was recorded. The process was repeated for every group with intermittent cleaning of the locomotion chambers using 70% alcohol in order to prevent olfactory cues left by the previous animal in the chamber.

Stereotype Behaviour Test

The methodology outlined by Arowona *et al.* [22] was employed to conduct the stereotype behaviour test, with little modification. One hour after pre-treatment with drugs, ketamine was administered to induce psychosis. Immediately after the five-minute of ketamine induction, the animals were individually placed into a transparent observation container and assessed for stereotype behaviours. Stereotype behaviours were recorded for 2 min at every 5, 10, 15, 20, 30, 45 and 60 min of the experiment. These behaviours were scored as 0 – no movement, 1 – presence of head movement, 2 – Intermittent sniffing action, 3 – chewing and 4 – licking action. The total stereotypy scores were determined according to the modified method of scoring by Bourin *et al.* [23]. Similarly, there was an intermittent cleaning of the transparent chambers using 70% alcohol.

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